Ultra-High Resolution Structured-Illumination Microscopy

NIST researchers have taken the recently developed structured-illumination microscopy and applied it using ultra-high 1.65 numerical aperture (NA) objectives to record sub-100 nm resolution images in the far field of single quantum dots and fluorescently-stained biological samples. Structured-illumination microscopy uses Moiré patterns to provide super-resolving imaging. By taking advantage of the aliasing effect that occurs when a sample is illuminated with a fine sinusoidal pattern, a synthetic image can be formed from a series of Moiré patterns that effectively doubles the resolution of the original image.

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Light microscopy is a widely used analytical tool because it provides non-destructive, real-time, three-dimensional imaging with chemically-specific contrast. However diffraction effects typically blur the resolution of these microscopes to 200 nm or worse, which limits their utility for the study of nanoscale materials.

The finest possible sinusoidal pattern that can be imaged onto a sample using a microscope objective has a pitch d given by:

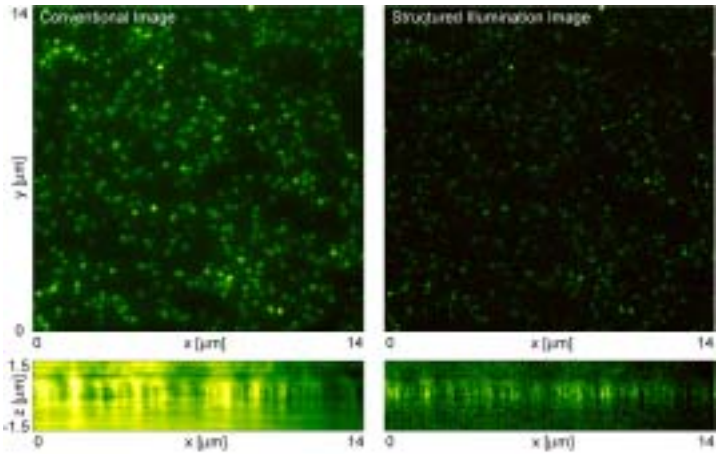
$\lambda_{ex} /(4\pi \cdot NA)$, where λ_{ex} is the excitation wavelength.

In principal, d could be made very small using ultraviolet wavelengths, but many fluorescent samples require visible or blue excitation light (nominally 500 nm), which means that increasing the NA is the only practical avenue to higher resolution. Recently, a new objective became available that uses high-optical index glass to achieve a 1.65 NA, which is 17% larger than was previously available.

NIST researchers developed a structured-illumination microscope based on a high-optical index glass objective that achieves a resolution of 75 nm using 488 nm excitation.

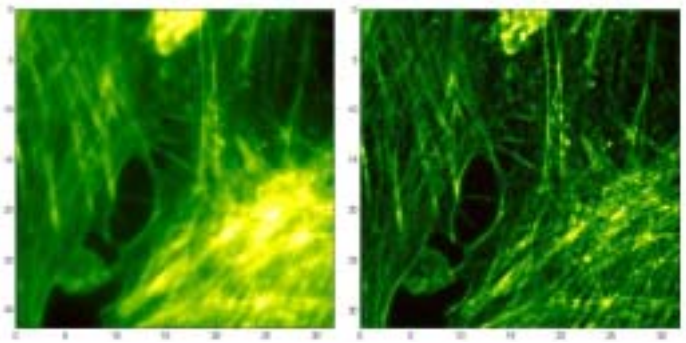
The resolution was measured using the full-width at half-maximum (FWHM) of single quantum dot images. Figure 1 shows a comparison between the conventional image, which has a FWHM of 195 nm, and the structured illumination technique with a FWHM of 75 nm.

Figure 1. ***Shown here are convention and superresolving fluorescence images of single green-emitting quantum dots taken using a 1.65 NA structured-illumination microscope.***



The bottom two panels in Figure 1 are side views of the image plane. The comparison between the conventional and structured-illumination images shows the improvement for both lateral and axial resolution.

Figure 2. ***Fluorescently stained bovine endothelia cells imaged conventionally and with the use of structured-illumination. The scale is provided in microns.***



The figure above shows the results of this technique for a biological sample. In this case, the optical sectioning along the z-axis can be of major help in rejecting out-of-focus light.

Future Plans: The fluorescence microscope is now developed to a point where it can be applied to problems in microbiology, and work is currently underway in the study of nanoparticle accumulation in bacteria. Of particular interest is the extension of resolution enhancement to contrast mechanisms based on intrinsic chemical contrast, like the spectroscopic fingerprint that spontaneous and coherent Raman scattering can provide for non-labeled samples. Generally speaking, these techniques require the use of a scanning laser microscope due to their use of high-excitation power levels. To meet this need, we are developing a hybrid technique that uses sinusoidal modulation of a laser beam during the raster-scanning image acquisition. Using this approach, it should be possible to provide sub-100 nm resolution at a cost of a three-time increase in image acquisition time over conventional imaging.

Publication in Preparation: A manuscript entitled "Benchmarking the Performance of Ultra-High Numerical Aperture Objectives for Structured Illumination Fluorescence Microscopy